

AMENDMENTS TO THE SPECIFICATIONIn the Specification

Please substitute the following amended paragraph(s) and/or section(s) (deleted  
5 matter is shown by strikethrough and added matter is shown by underlining):

Please substitute the following amended paragraph for the first paragraph, which was  
added by preliminary amendment of February 5, 2002:

Page 1, lines 5-8, This application is a divisional of U.S. application serial no.  
10 09/147,897 filed August 30, 1999 entitled "Methods and Devices for Preparing Protein  
Concentrates" filed under 35 U.S.C. §3.71 with priority to International Application No.  
PCT/US97/16897, filed September 22, 1997, which claims priority to U.S. application  
serial no. 60/026,532~~6~~ filed September 23, 1996; U.S. application serial no. 60/039,904  
filed March 4, 1997 and U.S. application serial no. 60/040,417 filed March 13, 1997, the  
15 disclosures of which are herein incorporated by reference.

Page 25, line 18-page 27, line 3

The polymeric crosslinkers may be prepared using variety of different synthetic  
methods. In a preferred embodiment, the polymer described in structure A can be  
20 obtained by a ring opening polymerization of trimethylene carbonate initiated by a  
dihydroxy compound such as polyethylene glycol molecular weight 2000 d in presence of  
a suitable catalyst such as stannous octoate. The hydroxy groups of the copolymer thus  
obtained are then activated with carbodiimidazole (CDI). The CDI activated polymer can  
then be reacted with a protein concentrate as prepared by this invention to form a  
25 crosslinked gel. The reaction conditions of the crosslinking reaction will depend on the  
nature of activating group employed. Preferred reactions are at pH 5 to 8, most preferred  
at pH 7.4. The resultant gel degrades due to hydrolysis of the biodegradable polymer

such as polytrimethylene carbonate polymer inside the crosslinker. The crosslinking density of the resultant network can be controlled by the overall molecular weight of the structure. A lower molecular weight such as 600 will give much higher crosslinking density as compared to a higher molecular weight crosslinker such a with molecular weight 10000 daltons. The high molecular weight linear crosslinker is preferred in obtaining elastic gels. The reaction between proteins and crosslinkers can be carried out directly on tissues and used as tissue glue. A trifunctional biodegradable crosslinker can be obtained by initiating a polymerization of lactide with trihydroxy polyethylene glycol (ethoxylated trimethylol propane triol) in presence of stannous octoate. The degree of polymerization of lactide is kept less than 5. This is achieved by choosing a molar ratio of PEG with lactide (molar ratio of lactide to PEG is 6, 2 per branch). The PEG-lactate trifunctional polymer is isolated. The hydroxy end groups of copolymers are then activated with CDI. Since this a trifunctional crosslinker, it will give higher crosslinking density as compared to similar molecular weight difunctional crosslinker. This gives additional flexibility in controlling crosslinking density of a crosslinked structure and hence their mechanical and biodegradation properties. The tertafunctional structures are obtained by reacting the polyalkylene oxide copolymer such as ~~Tetronic~~ TETRONIC 908 (obtained from BASF corporation) with caprolactone in presence of stannous octoate. The reaction is carried out in melt at 180° C for 6 hours under nitrogen atmosphere. The molar ratio of caprolactone to ~~Tetronic~~ TETRONIC 908 is kept to 12, which maintains water solubility of ~~Tetronic~~ TETRONIC 908-caprolactone copolymer in water. The polymer is activated with CDI and used in crosslinking reaction with proteins.

Page 35, line 6-line 12.

25 Polyethylene glycols (Merck, mol. wt 20000 and BDH mol. wt 6000) were used as received. ~~Pluronic~~ PLURONIC® and ~~Tetronic~~ TETRONIC® polyols were purchased from BASF corporation. Photoinitiator ~~Darocure~~ DAROCURE® 2959 was purchased

from Ciba Geigy. The biodegradable polymers like polylactic acid, polyglycolic acid are purchased from Polysciences. All other reagents, solvents are of reagent grade and are purchased from commercial sources such as Fluka, Aldrich and Sigma. Small laboratory equipment was purchased from Fisher or Cole-Parmer.

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Page 36, line 17- page 37, line 5

50 grams of ~~Pluronic~~ PLURONIC<sup>®</sup> F127 (a PEO-PPO-PEO block copolymer with 70% PEO content, molecular weight 12500 daltons, purchased from BASF corporation) is dried under vacuum at 80-100° C. The polymeric diol is then transferred  
10 to a 3 neck reaction flask equipped with nitrogen inlet and thermometer. 500 ml toluene, 3.3 ml of triethylamine amine and 1.9 ml of acryloyl chloride are added to the reaction mixture under dry nitrogen atmosphere. The reaction mixture is stirred overnight and filtered to remove triethylamine hydrochloride. The filtrate is added to 3000 ml hexane to precipitate the diacrylate derivative of ~~Pluronic~~ PLURONIC F127. The  
15 macromonomer is purified by several dissolution-precipitation steps from THF-hexane solvent-nonsolvent system. Finally the diacrylate is dried under vacuum at 40° C to a constant weight.

Other derivatives of ~~Pluronics~~ PLURONICS with different arrangement of PEO-PPO blocks and with different HLB values can also be acrylated in a similar manner.

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Page 39, line 1-line 9

30 g of ~~Tetronic~~ TETRONIC 908 polyol is dissolved in 400 ml dry benzene. 100 ml of benzene is distilled to remove traces of water from the polyol. The solution is cooled to 30° C and 1.45 g triethylamine and 1.90 g acryloyl chloride are added. The  
25 reaction mixture is refluxed for 1 h under argon atmosphere. It is then cooled and then filtered to removed triethylamine hydrochloride. The filtrate is then added to 2000 ml hexane to precipitate the polymer. The polymer is purified by several precipitations from

THF-hexane solvent-nonsolvent system. Further solvent removal/drying is achieved by vacuum drying overnight at 60° C.

Page 43, line 20-page 44, line 6

5        Synthesis of ~~Tetronic~~ TETRONIC 908-caprolactone polyol(PCLP)-30 g of ~~Tetronic~~ TETRONIC 908 is charged in a dry 3 neck flask equipped with magnetic stirrer and vacuum inlet. The flask is then heated in a silicone oil bath at 100° C for 12 hours to dry the ~~Tetronic~~ TETRONIC 908. The flask is cooled to room temperature and 1.642 g of caprolactone and 0.02 g of stannous 2-ethylhexanoate are added to the flask. The flask  
10 is heated to 180° C for 6 hours under nitrogen atmosphere. The reaction is product is then dissolved in 200 ml dry toluene (warming of toluene accelerates dissolution). The toluene solution is added to 2,000 ml dry heptane with constant stirring. The product is isolated by filtration. Further purification is accomplished by precipitation of toluene solution of PCLP in heptane. The product is dried in vacuum at 40° C and used  
15 immediately in the activation reaction.

Page 44, line 9-line 14

30 g of ~~Tetronic~~ TETRONIC 908-caprolactone copolymer is dissolved in 400 ml dry benzene. About 100 ml of benzene is distilled off and the solution is cooled to 50° C.  
20 2.24 g of carbodiimidazole is added to reaction mixture. The mixture is refluxed for 2 h under nitrogen atmosphere. At the end of 2 hour period, the solution cooled added to 4,000 ml hexane to precipitate the polymer. It is further purified by repeated (3 times) precipitation from toluene-hexane system. The polymer is dried under vacuum at 40° C.

Page 44, line 17-line 23

30 g of ~~Tetronie~~ TETRONIC 908-caprolactone copolymer is dissolved in 400 ml dry benzene. About 100 ml of benzene is distilled off and the solution is cooled to 50° C. 2.50 g of succinic anhydride is added to reaction mixture. The mixture is refluxed for 5 hours under nitrogen atmosphere. At the end of 5 hour period, the solution cooled and then added to 4,000 ml hexane to precipitate the polymer. It is further purified by repeated (3 times) precipitation from toluene-hexane system. The polymer is dried under vacuum at 40° C.

10 Page 45, line 4-line 9

10 g of ~~Tetronie~~ TETRONIC 908-caprolactone succinate prepared by method described above is dissolved in 100 ml dry methylene chloride. The mixture is cooled to 0° C in ice bath and 0.5 g of 4-dimthylaminopyridine and 1 g of dicyclohexylcarbodiimide (DCC) are added. The mixture is stirred at 0° C for 6 hour and filtered. The filtrate is then added to 2,000 ml dry hexane to precipitate the activated succinimydyl ester. The product is isolated by filtration, dried under vacuum and stored under argon at 4° C.